processing diverse chemical compounds. For the production of compounds within industrial bioreactors, it is optimal to have a uniform dispersal of bacterial cells within solution, but mutations in bacterial cells may generate surface properties leading to cell aggregation. During long-term culture in the laboratory, mutants of the bacterium A. baylyi ADP1 originated a distinctive phenotype of cell aggregation. Genome sequencing and the analysis of gene knockouts showed this aggregation to be due to mutations in the per and pgi genes, and a reduction in bioemulsifier production. Qualitative analysis of Atomic Force Microscopy (AFM) visualizations identified altered appearances of cell surfaces correlating with the difference in cell aggregation phenotype. AFM force spectroscopy experiments were then conducted to compare the adhesive and viscoelastic properties of aggregating cells to non-aggregating cells. The most distinctive difference found for force spectroscopy measurements was for a four-fold difference in nN in adhesion that was attributable to pgi. Overall, this experiment has resulted in a multilevel approach for the evaluation and detection of a cell aggregation phenotype in mutant strains of A. baylyi ADP1.

**2026-Pos Board B163**

**Membrane Environment can Enhance the Interaction of Glycan Binding Protein to Cell Surface Glycan Receptors**

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The binding of lectins to glycan receptors on the host cell surface is a key step contributing to the virulence and species specificity of most viruses. This is exemplified by the viral protein hemagglutinin (HA) of the influenza A virus, whose binding specificity is modulated by the linkage pattern of terminal sialic acids on glycan receptors of host epithelial cells. Such specificity dictates whether transmission is confined to a particular animal species or jumps between species. Here we show, using H5N1 avian influenza as a model, that the specific binding of recombinant HA to $\alpha$2-3 linked sialic acids can be enhanced dramatically by interaction with the surface of the lipid membrane. This effect can be quantitatively accounted for by a two-stage process in which weak association of HA with the membrane surface precedes more specific and tighter binding to the glycan receptor. The weak protein-membrane interaction discovered here in the model system may play an important secondary role in the infection and pathogenesis of the influenza A virus.

**2027-Pos Board B164**

**Impact of Composition upon Ordered Membrane Domain("Raft") Formation by Lipids from Pathogenic Bacteria**

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Co-existing ordered (raft) and disordered membrane domains have been indentified in the outer membrane of the pathogenic bacterium Borrelia burgdorferi, the bacterium which causes Lyme disease. Co-existing ordered and disordered membranes can also be detected into B. burgdorferi lipid extracts. However, unlike eukaryotic cells, B. burgdorferi lack sphingolipids, which are crucial component of eukaryotic rafts. In order to understand the basis of domain formation in this organism we have isolated the major lipids of B. burgdorferi by thin layer chromatography, and have initiated studies of their physical properties when dispersed in aqueous solutions. We have found that mixtures of the predominant lipids found in B. burgdorferi, namely, ACGal, a lipid in which a fatty acyl chain and cholesterol are linked to galactose, monogalactosyldiglyceride (MGalD) with phosphatidylcholine (PC) can form ordered domains with thermal stabilities similar to that in whole lipid extracts. However, for individual lipid aqueous dispersions domain formation and/or stability is very different than in whole lipid extracts. Combinations of B. burgdorferi lipids are being studied to identify which lipid are necessary and sufficient for the formation of co-existing ordered and disordered domains in this bacterium and related ones.

**2028-Pos Board B165**

**Stabilization of Glycosphingolipid Domains by Palmitoyl Ceramide in Unsaturated Phosphatidylcholine Bilayers**

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Ceramides and glycosphingolipids (GSLs) are minor components in most eukaryotic cells. Since ceramides may be generated in lipid raft like domains by enzyme degradation of sphingomyelin (SM), ceramide/GSL interactions may become relevant in cell membranes. To examine their mutual interactions, we have prepared binary and ternary model bilayer systems composed of a disordered lipid (unsaturated phosphatidylcholine), and different combinations of saturated sphingolipids (palmitoyl SM, palmitoyl ceramide (PCer), and hydroxylated or non-hydroxylated galactosyl or glucosyl palmitoyl-ceramide (PGalCer or PGlcCer)). We have used trans-parinaric acid (tPA) as a probe to detect the ordered domains formed by the sphingolipids in the phosphatidylcholine bilayer. In binary systems, the PCer formed the most thermostable ordered domains, followed by PGalCer, OH-PGalCer, OH-PGlcCer, and PGlcCer. The PSM domains were the least thermostable. Addition of PCer to the GSL or PSM domains increased their thermostability, with the exception of PGalCer, whose thermostability was unaffected by inclusion of PCer. Lifetime analysis of tPA suggested that all sphingolipid ordered domains became even more ordered in the presence of PCer. We conclude that PCer was able to interact with all the examined sphingolipids and increased packing order in the domains.

**2029-Pos Board B166**

**Comparison of Line Tension Measurement Techniques in Phase Separated Multi-Component Lipid Monolayers**

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Langmuir monolayers of multi-component lipid compositions have been used to study the mixing behavior of sterol-phospholipid systems. Using traditional Langmuir pressure-area isotherms and fluorescence microscopy techniques we compare line tension measurements using two methods of image analysis. Line tension between coexisting phases of sterol-rich and sterol-poor domains can be extracted from a Fourier analysis of domain boundary fluctuations (J. Phys. Chem. B, 111:11091-11094). These measurements will be compared to a recently developed non-perturbative technique based on domain size distribution (Proc. Natl. Acad. Sci. 110:13272-1327). Until now these two measurement techniques have not been compared on the same data set. The compositions studied include 30:70 mixtures of cholesterol and DMPC, DLPC, and DCPC. As well as 25:75 mixtures of 25-hydroxycholesterol DMPC systems.

**2030-Pos Board B167**

**The Average Area Per Molecule of Cholesterol/PC-Lipid Bilayers: A Review of Experimental Data and a Physically Inspired Model**

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We recently documented that beta-cyclodextrin extracts cholesterol at different rates from supported lipid bilayers containing either DMPC, SOPC, or DOPC [Litz & Keller, BJ, 2013, 93A]. Quantitative measurement of the rate of cholesterol depletion relies on accurate knowledge of the average area per molecule within each bilayer, as does calibration of fixed-area molecular dynamic simulations [e.g. Klauda & Nagle, BJ, 2006, 2796]. A challenge is to integrate a plethora of seemingly incompatible experimental results, which yield significantly different average areas per molecule of PC-lipid/cholesterol bilayers. Historically, disagreements between values derived from x-ray and neutron scattering have been attributed to differences in sensitivity between the two techniques, and more recent approaches have analyzed scattering data from both techniques [e.g. Kucerka & Katsaras, BJ, 2008, 2356]. Here I show that the majority of the data from which areal measurements are derived is in agreement, and that most disparity in reported values arises from the choice of difficult-to-measure physical parameters. I provide an estimate of the uncertainty of how the area of a PC-lipid bilayer changes as a function of the mole fraction of cholesterol and derive a physically-inspired, two-parameter model to predict the change. I compare the efficacy of my model with that of the currently preferred four-parameter model [Edholm & Nagle, BJ, 2005, 1827]. I then apply my results to quantitatively report rates of cholesterol depletion from two-component lipid bilayers.

**2031-Pos Board B168**

**Cholesterol Bilayer Domain in Phospholipid Bilayer Membranes can be Detected by Confocal Microscope**

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The unique feature of the eye lens fiber-cell plasma membrane is its extremely high cholesterol content; cholesterol/phospholipid molar ratio can be as high as

4 in human lens nucleus. Cholesterol saturates bulk phospholipid bilayers and induces formation of immiscible cholesterol bilayer domains (CBDs) within membranes. The presence of CBDs plays crucial role ensuring that surrounding phospholipid bilayers are saturated with cholesterol which keeps the bulk physical properties of lens-lipid membranes consistent and independent of changes in phospholipid composition. Thus, CBDs help to maintain lens-membrane homeostasis when the membrane phospholipid composition changes significantly with age. Our previous experiments were based on electron paramagnetic resonance spin-labeling methods. They allowed to detect CBDs in model and lens lipid membranes at high cholesterol content (1,2). In current experiments the giant unilamellar vesicles made of cholesterol/distearoylphosphatidylcholine (Chol/DSPC) mixtures, with mixing ration from 0.5 to 5 and labeled with phospholipid analog (1,1′-Dioctadecyl-3,3,3',3′-Tetramethylindocarbocyanine-5,5′-Disulfonic Acid) and cholesterol analog (23-(dipyrromethemeboron difluoride)-24-norcholesterol) fluorescent probes, were prepared using the electroformation method (3). Confocal microscopy experiment allowed us to confirm that membranes with higher Chol/DSPC molar ratio contain two distinct lipid environments, the bulk phospholipid-cholesterol domain (PCD) and the CBD. The amount of CBD was greater in membranes with higher Chol/DSPC ratio. According to these results, CBD is always located on the top of the giant vesicle. Rationale for this discovery is that CBD has smaller mass per unit area of the vesicle compared with PCD. Additionally, it can be concluded that vesicles with higher Chol/DSPC molar ratio are more rigid and show less wobbling motion in water environment.

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**2032-Pos Board B169**

**Correlated Motion and Complex Formation of Lipid-Raft Components Analyzed by High-Resolution Secondary Ion Mass Spectrometry**

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It is widely believed that certain membrane lipids and membrane-anchored proteins associate to form clusters with emergent function. While extensive data on phase diagrams are available for a range of lipid compositions, these are most commonly visualized by the partitioning of dyes between different phases. We have been developing the use of secondary ion mass spectrometry imaging to directly visualize molecules of interest within supported lipid bilayers (Lozano et al. JACS 2013). Specifically, we want to address the question which neutral membrane components associate with the well-known lipid-raft marker ganglioside GM1, which has a single negative charge, after GM1 is reorganized by an in-plane electric field on a patterned supported bilayer (SLB). NanoSIMS imaging was used to generate molecule-specific concentration profiles of several SLB compositions following electrophoresis: GM1/DOPC, GM1/CHOL/DOPC, and GM1/CHOL/PSM/DOPC. In a control SLB sample without GM1 it is observed that CHOL, PSM, and DOPC do not reorganize by membrane electrophoresis. However, NanoSIMS images clearly illustrate that PSM and CHOL associate with GM1 as it is transported toward the positive electrode while DOPC is displaced in the opposite direction towards the negative electrode. Further analysis of the concentration profiles for the co-diffusing components (GM1/CHOL/PSM) suggest the formation of a 4:2:1 PSM:GM1:CHOL stoichiometric complex. These results demonstrate that both cholesterol and sphingomyelin do tend to associate with GM1 and we might speculate that similar behavior would be observed for GPI or myristolyated proteins.

**2033-Pos Board B170**

**Domain Morphologies of Complex Phosphoinositide/Lipid Langmuir Films in the Presence of Bivalent Cations**

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Although phosphatidylinositol (PI) and phosphoinositides (PIPs) only comprise a small percentage of the inner leaflet of the plasma membrane, they mediate a large variety of signaling events. In previous studies, we have observed the absence of macroscopically discernible domains in mixtures of PI/PC and PI(4,5)P2/PC. The addition of cholesterol to these mixtures results in condensation of the monolayer and hence domain formation. To better mimic the ionic conditions and hydrogen bonding properties of the inner leaflet plasma membrane, we investigated in this study the effect of common inner leaflet plasma membrane lipids like phosphatidylethanolamine (PE), phosphatidylserine (PS) and PI, on phosphoinositide domain behavior in the presence of cholesterol and/or bivalent cations. We find that the addition of varying concentrations of $Ca^{2+}$ to PI(4,5)P2/cholesterol monolayer leads to a size reduction of the domains. We hypothesize that this is due to a penetration of the $Ca^{2+}$ ions into the PI(4,5)P2 headgroup region, leading to a disruption of the hydrogen bond network formed by the PI(4,5)P2 headgroup and cholesterol. We find for PE/PI(4,5)P2 and PI/PI(4,5)P2 homogeneous mixing of the monolayer. The addition of cholesterol to PE/PI(4,5)P2 leads to the formation of small domains at low surface pressures that disappear at higher pressures. In addition to cholesterol, we also investigate in this study the effect of bivalent cations on these lipid mixtures.

**2034-Pos Board B171**

**Ceramide and Cholesterol Effects on Phospholipid Bilayers under the AFM: Characterization of Complex Lipid Phases**

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Atomic force microscopy (AFM) has been applied to the characterization of palmitoylceramide (pCer) and cholesterol (Chol) incorporation into phospholipid-based supported planar bilayers (SPBs) at 22°C. Phospholipids were dipalmitoyl phosphatidylcholine (DPPC) or palmitoyl sphingomyelin (pSM). Membranes of different compositions were prepared by either the vesicle adsorption or the spin-coating procedures (the latter strictly for pCer-containing mixtures) and analyzed with a combination of AFM imaging (domain segregation, bilayer thickness and roughness) and force spectroscopy (mechanical resistance to indentation). The mixtures under study were pure phospholipids (pSM, DPPC), phospholipid:Chol (70:30), phospholipid:-Chol:pCer (2:1:1) and phospholipid:pCer (90:10, 80:20 and 70:30). Binary phospholipid:pCer mixtures at increasing ceramide ratios gave rise to highly-resistant segregated domains with increasing extension but similar properties in terms of breakthrough forces, thicknesses and roughnesses. These ceramide-enriched domains are able to exclude a fluorescent lipid probe (DiIC18) due to their high intermolecular packing. Interestingly, these domains have been reported to disappear when model membranes become highly enriched in cholesterol in fluid membranes or in the absence of a fluid phase (our case). Indeed, the ternary mixtures (2:1:1) gave rise to a homogenous lamellar gel phase with significantly different properties when compared to all of the other mixtures under study: ternary mixtures showed a reduced thickness and an intermediate roughness and mechanical resistance when compared to phospholipid:Chol (70:30) and phospholipid:pCer. These differences were statistically significant. More importantly, at those relatively high pCer and Chol concentrations in ternary mixtures, no mutual displacement of these molecules was observed. The data become relevant in the context of sphingolipid signaling and membrane platform formation.

**2035-Pos Board B172**

**End-Product Diacylglycerol Enhances Activity of Phosphatidylinositol Phospholipase C through Changes in Membrane Lipid Domain Structure**

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DAG-induced activation of PI-PLC has been studied using as substrates vesicles containing PI, either pure or in mixtures with DMPC, DSPC, sphingomyelin, or galactosylceramide. At 22°C DAG at 33 mol% increases PI-PLC activity in all the mixtures, but not in pure PI bilayers. DAG also causes an overall decrease in DPH polarization (decreased molecular order) in all samples, and increased overall enzyme binding. Confocal fluorescence microscopy examination of GUV of all the compositions under study, with or without DAG, and quantitative evaluation of the phase behaviour using LAURDAN generalized polarization, and of enzyme binding to the various domains, indicate that DAG activates PI-PLC whenever it can generate fluid domains to which the enzyme can bind with high affinity. In the specific case of PI:DMPC bilayers at 22°C DAG induced increased enzyme binding and activation, but no microscopic domain separation was observed, the presence of DAG-generated nanodomains is proposed instead for this system. In PI:galactosylceramide mixtures DAG may exert its activation role through the generation of small vesicles, that PI-PLC is known to degrade at higher rates. In general our results indicate that global measurements using fluorescent probes in vesicle suspensions in cuvette are not enough to understand DAG effects that take place at the domain level. The above data reinforce the idea of DAG as an important physical agent regulating membrane or cell properties.